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# The anti-ischemic effects of CP-060*S* during pacing-induced ischemia in anesthetized dogs

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**Abstract**

CP-060*S*, (−)-(*S*)-2-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-3-[3-[*N*-methyl-*N*-[2-(3,4-methylenedioxyphenoxy)ethyl]-amino]propyl]-1,3-thiazolidin-4-one hydrogen fumarate, is a novel cardioprotective drug which prevents $Na^+$-, $Ca^{2+}$-overload and has $Ca^{2+}$ channel blocking activity. We compared the anti-ischemic effects of CP-060*S* with those of diltiazem, a $Ca^{2+}$ channel blocker, and R56865, *N*-[1-[4-(4-fluorophenoxy)butyl]-4-piperidinyl]-*N*-methyl-2-benzothiazolamine, a $Na^+$-, $Ca^{2+}$-overload inhibitor, in a canine pacing-induced ischemia model. CP-060*S* 100 μg $kg^{-1}$ significantly suppressed the pacing-induced ischemic epicardial ST-segment elevation by maximally 75%, while diltiazem 100 μg $kg^{-1}$ suppressed it by maximally 35%. R56865 100 μg $kg^{-1}$ significantly suppressed the ST-segment elevation by maximally 30%. In addition, diltiazem 100 μg $kg^{-1}$ caused synergistic suppression of ST-segment elevation by 70% when administered simultaneously with R56865 100 μg $kg^{-1}$. These results suggest that a $Na^+$-, $Ca^{2+}$-overload preventive action and a $Ca^{2+}$ channel blocking action independently contribute to the suppression of the ST-segment elevation. Therefore, CP-060*S* may suppress pacing-induced ST-segment elevation by a dual action by preventing $Na^+$-, $Ca^{2+}$-overload and the $Ca^{2+}$ channel blockade. 

*Keywords:* CP-060*S*; Diltiazem; R56865; Ischemia; Pacing $Na^+$-, $Ca^{2+}$-overload; $Ca^{2+}$ channel blocker

## 1. Introduction

Angina pectoris is caused by the myocardial ischemia that results from a decrease in oxygen supply and an increase in oxygen demand (Opie, 1991). $Ca^{2+}$ channel blockers, e.g., diltiazem, nifedipine, etc., are thought to improve this oxygen supply–demand imbalance by causing coronary vasodilation, and by exerting negative chronotropic and inotropic effects. Direct cardioprotective drugs, e.g., ranolazine, trimetazidine, etc., have been recently reported to be efficacious against angina pectoris (Cocco et al., 1992; Detry et al., 1994). Therefore, a drug which possesses a $Ca^{2+}$ channel blocking effect along with direct cardioprotective effects would be of considerable benefit to patients with angina pectoris.

A rapid intracellular accumulation of $Na^+$ occurs during myocardial ischemia and is attributed to intracellular acidosis and ATP depletion (Butwell et al., 1993; Van Emous et al., 1997). Lidocaine reduces this $Na^+$-overload and the intracellular acidosis, and also protects against the ATP depletion (Butwell et al., 1993; Van Emous et al., 1997). Therefore, $Na^+$ channels play an important role in the $Na^+$-overload early in the course of myocardial ischemia. Recently, hypoxia (Ju et al., 1996) and the ischemic metabolite lysophosphatidylcholine (Burnashev et al., 1991) have been reported to induce long-lasting bursts of channel opening and to delay the inactivation of the $Na^+$ channels. This non-inactivating $Na^+$ current has been proposed to be one of the pathways for $Na^+$-overload in the ischemic heart (Ver Donck et al., 1993). Indeed, R56865, a $Na^+$-, $Ca^{2+}$-overload inhibitor which inhibits the non-inactivating $Na^+$ current, is able to suppress the ST-segment elevation during myocardial ischemia in anesthetized rabbits (Verscheure et al., 1995), and to prevent left ventricular diastolic disorder during pacing-induced ischemia in anesthetized dogs (Vandeplassche et al., 1991b).

CP-060*S*, (−)-(*S*)-2-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-3-[3-[*N*-methyl-*N*-[2-(3,4-methylenedioxy-

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phenoxy)ethyl]amino]propyl]-1,3-thiazolidin-4-one hydrogen fumarate, is a novel cardioprotective drug which prevents $Na^+$-, $Ca^{2+}$-overload and has $Ca^{2+}$ channel blocking activity (Tamura et al., 1996; Ohya et al., 1997; Suzuki et al., 1998a). Similar to R56865, CP-060*S* inhibits the non-inactivating $Na^+$ current without apparently suppressing physiological $Na^+$ channel activity, consequently preventing $Na^+$-, $Ca^{2+}$-overload (Fukazawa et al., 1997). By the combined effect of preventing the $Na^+$-, $Ca^{2+}$-overload and the $Ca^{2+}$ channel blockade, CP-060*S* reduces myocardial infarct size in anesthetized dogs (Suzuki et al., 1998b) and inhibits ischemia- and reperfusion-induced arrhythmias in anesthetized rats (Koga et al., 1998). As a result of causing $Ca^{2+}$ channel blockade, CP-060*S* decreases myocardial oxygen consumption in anesthetized dogs (Suzuki et al., 1999), and suppresses the ST-segment changes in a rat methacholine-induced or a rat vasopressin-induced vasospastic angina model (Fukazawa et al., 1998; Adachi et al., 1998). Although it is reported that the CP-060*S*-induced suppression of the ST-segment changes is longer lasting than its cardiosuppressive effects in a canine pacing-induced ischemia model (Sugiyama and Hashimoto, 1998), the contribution of $Na^+$-, $Ca^{2+}$-overload to the reversible myocardial ischemia such as occurs in angina pectoris remains to be elucidated.

In this study, we compared the anti-ischemic effects of CP-060*S* with those of diltiazem, a $Ca^{2+}$ channel blocker, and R56865, a $Na^+$-, $Ca^{2+}$-overload inhibitor, in a canine pacing-induced ischemia model to clarify the mechanisms of the anti-ischemic effects of CP-060*S*.

## 2. Material and methods

The animals used in this experiment were treated in accordance with Chugai Pharmaceutical's ethical guidelines of animal care, handling, and termination.

### *2.1. Animal preparation*

Male beagle dogs (9.2–13.2 kg) were anesthetized with sodium pentobarbital (35 mg $kg^{-1}$ intravenously) and anesthesia was maintained by intravenous infusion of sodium pentobarbital (5 mg $kg^{-1}$ $h^{-1}$). After endotracheal intubation, artificial respiration was performed by means of a respiration pump (model 607D, Harvard, South Natick, MA, USA) with room air. Respiratory rate and stroke volume were adjusted to maintain arterial blood gases within the normal physiological range. Polyethylene catheters were inserted in the right femoral vein for drug administration and in the left femoral artery for measurement of mean arterial pressure. Mean arterial pressure was measured with a pressure transducer (model DX-300, Nihon Kohden, Tokyo, Japan), and heart rate was measured using a tachometer (model AT-601G, Nihon Kohden, Tokyo, Japan) triggered by the pulse wave of the mean arterial pressure. Left ventricular pressure was measured with a 5F micro-tip catheter transducer (model SPC-350, Millar, Houston, TX, USA) introduced into the left ventricle via the right femoral artery. Maximal first derivative of left ventricular pressure (LVdP/dtmax) was calculated with a differentiator (model EQ-600G, Nihon Kohden, Tokyo, Japan). The thoracotomy was performed through the left fifth intercostal space. The pericardium was opened and reflected to form a cradle for suspending the heart. The left anterior descending coronary artery was dissected at the origin, and a screw-type occluder (model 1933, MT Giken, Tokyo, Japan) was placed around it to cause partial occlusion of the left anterior descending coronary artery. A pulsed doppler flow probe (model PD-20, Crystal Biotech, Hopkinton, MA, USA) was placed around the left anterior descending coronary artery distal to the occluder to monitor left anterior descending coronary artery flow. Another flow probe was placed around the left circumflex coronary artery proximal to the first major obtuse marginal branch to monitor left circumflex coronary artery flow. The myocardial ischemic changes were determined by means of monopolar epicardial leads (Unique Medical, Tokyo, Japan) sutured to the surface of the left ventricle supplied by the left anterior descending coronary artery. The leads from each electrode were connected to a selector box (model PB-680G, Nihon Kohden, Tokyo, Japan) that allowed rapid individual sampling of all sites. The epicardial electrocardiograms (ECG) were recorded using a bioelectric amplifier (model AB-621G, Nihon Kohden, Tokyo, Japan). For pacing of the heart, a bipolar silver electrode was sutured to the left auricular appendage. Atrial pacing was accomplished with a driving stimulus of 1 ms duration, with a 1-ms delay, at 3–5 V, delivered by a stimulator (model SEN-7203, Nihon Kohden, Tokyo, Japan).

### *2.2. Experimental protocol*

After the completion of surgery, the preparation was allowed to stabilize. Baseline hemodynamic function and

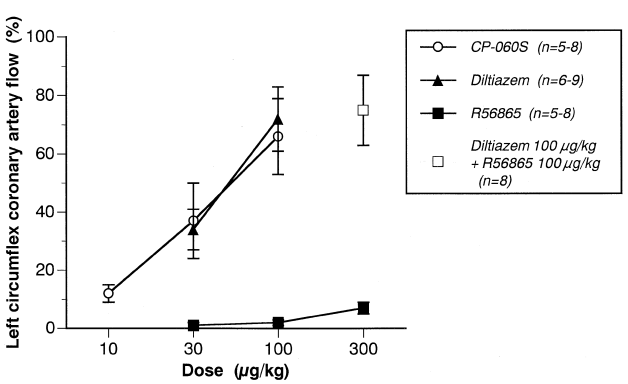


Fig. 1. Effects of CP-060*S*, diltiazem, R56865, and co-administration of diltiazem 100 μg $kg^{-1}$ and R56865 100 μg $kg^{-1}$ on the maximal changes in left circumflex coronary artery flow in the presence of a partial left anterior descending coronary artery stenosis immediately after the administration of the drugs to anesthetized dogs.

Table 1
Hemodynamic variables in the vehicle group during pacing-induced ischemia in anesthetized dogs

| | Baseline | Stenosis | Pacing after drug treatment | | | |
|---|---|---|---|---|---|---|
| | | | Control | 30 min | 60 min | 90 min |
| Heart rate (beats $min^{-1}$) | $151 \pm 4$ | $149 \pm 5$ | $232 \pm 4^{b}$ | $232 \pm 5^{b}$ | $232 \pm 4^{b}$ | $232 \pm 4^{b}$ |
| Mean arterial pressure (mm Hg) | $104 \pm 3$ | $103 \pm 3$ | $98 \pm 4$ | $96 \pm 4^{a}$ | $97 \pm 3^{a}$ | $98 \pm 4$ |
| Rate–pressure product (mm Hg min′$1000^{-1}$) | $15.7 \pm 0.6$ | $15.4 \pm 0.6$ | $22.7 \pm 0.9^{b}$ | $22.2 \pm 0.9^{b}$ | $22.5 \pm 0.7^{b}$ | $22.6 \pm 0.7^{b}$ |
| Left anterior descending coronary artery flow (ml $min^{-1}$) | $8 \pm 1^{b}$ | $6 \pm 1$ | $5 \pm 1$ | $6 \pm 1$ | $7 \pm 1$ | $6 \pm 1$ |
| Left circumflex coronary artery flow (ml $min^{-1}$) | $22 \pm 5$ | $24 \pm 5$ | $35 \pm 7^{b}$ | $34 \pm 7^{b}$ | $33 \pm 6^{b}$ | $33 \pm 5^{b}$ |
| LVEDP (mm Hg) | $4.06 \pm 0.24$ | $4.11 \pm 0.23$ | $5.28 \pm 0.63^{a}$ | $4.86 \pm 0.64$ | $5.19 \pm 0.36^{a}$ | $4.95 \pm 0.34$ |
| LVdP/dtmax $\times 10^{-3}$ (mm Hg $s^{-1}$) | $2.5 \pm 0.1^{b}$ | $2.2 \pm 0.1$ | $2.1 \pm 0.1$ | $2.0 \pm 0.1$ | $2.0 \pm 0.1$ | $2.0 \pm 0.1$ |

Values are expressed as means ± S.E.M. LVEDP: left ventricular end-diastolic pressure. LVdP/dtmax: maximal first derivative of left ventricular pressure.
[a] $P < 0.05$. vs. stenosis.
[b] $P < 0.01$ vs. stenosis.

ECG data were recorded. The left anterior descending coronary artery flow was gradually reduced by the screw clamp until a critical degree of stenosis was achieved, as demonstrated by the failure of a 10-s total left anterior descending coronary artery occlusion to evoke a hyperemic response. Once the stenosis was established, it was maintained throughout the rest of the experiment with alternating periods of pacing. After the partial occlusion, a stabilization period followed. The heart was paced for 5 min at a rate 80–90 beats $min^{-1}$ above the baseline heart rate to obtain ischemic ECG changes. The ST-segment elevation data from a lead which was selected before drug treatment were used. At 30 min after the first pacing run, dogs received an intravenous injection of vehicle or a compound. Each successive 5-min pacing episode in a given experiment was performed 30, 60 and 90 min after drug treatment.

### 2.3. Regional myocardial blood flow

In another series of experiments, the effect of CP-060*S* on regional myocardial blood flow during pacing-induced ischemia was examined. The experimental protocol was performed as described above, with the following modifications: the successive 5 min pacing episodes were performed 60 min after drug treatment. Regional myocardial blood flow was measured with 15 μm colored microspheres (Dye-Trak, Triton Technologies, San Diego, CA, USA) by the reference withdrawal method as described previously (Suzuki et al., 1998b). Five injections of microspheres were made in each experiment (baseline, before control pacing, 3.5 min of control pacing, before pacing 60 min after drug treatment, and 3.5 min of pacing 60 min after drug treatment), each time with one of five colors (violet, white, yellow, blue, or red).

### 2.4. Drugs

CP-060*S*, (−)-(*S*)-2-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-3-[3-[*N*-methyl-*N*-[2-(3,4-methylenedioxyphenoxy)ethyl]amino]propyl]-1,3-thiazolidin-4-one hydrogen fumarate, and R56865, *N*-[1-[4-(4-fluorophenoxy)butyl]-4-piperidinyl]-*N*-methyl-2-benzothiazolamine, were synthesized at Fuji Gotemba Research laboratories, Chugai Pharmaceutical (Shizuoka, Japan). Diltiazem hydrochloride was obtained from Sigma (St. Louis, MO, USA). Drugs were dissolved in 0.01 N HCl.

### 2.5. Statistics

Intergroup differences were analyzed by analysis of variance (ANOVA) followed by post-hoc analysis using Dunnett's test or Tukey–Kramer's test. Intragroup differences were analyzed by repeated measures ANOVA followed by contrast analysis. Data are expressed as means ± S.E.M. Values of $P < 0.05$ are considered statistically significant.

## 3. Results

Fig. 1 summarizes the effects of CP-060*S*, diltiazem, R56865, and co-administration of diltiazem 100 μg $kg^{-1}$ and R56865 100 μg $kg^{-1}$ on the maximal changes in left circumflex coronary artery flow in the presence of partial left anterior descending coronary artery stenosis immedi-

Table 2
ST-segment at baseline, stenosis, control pacing in the different groups in anesthetized dogs

| | Baseline | Stenosis | Control pacing |
|---|---|---|---|
| ST-segment (mV) | | | |
| Vehicle ($n = 9$) | $0.3 \pm 0.2$ | $0.4 \pm 0.3$ | $8.7 \pm 1.0^{a}$ |
| CP-060*S* 10 μg $kg^{-1}$ ($n = 5$) | $0.5 \pm 0.2$ | $0.4 \pm 0.3$ | $8.2 \pm 0.6^{a}$ |
| CP-060*S* 30 μg $kg^{-1}$ ($n = 6$) | $0.3 \pm 0.3$ | $0.7 \pm 0.3$ | $8.2 \pm 0.6^{a}$ |
| CP-060*S* 100 μg $kg^{-1}$ ($n = 8$) | $0.4 \pm 0.1$ | $0.3 \pm 0.3$ | $8.1 \pm 0.8^{a}$ |
| Diltiazem 30 μg $kg^{-1}$ ($n = 6$) | $0.3 \pm 0.3$ | $0.4 \pm 0.4$ | $8.6 \pm 0.9^{a}$ |
| Diltiazem 100 μg $kg^{-1}$ ($n = 9$) | $0.3 \pm 0.3$ | $0.4 \pm 0.3$ | $8.4 \pm 0.7^{a}$ |
| R56865 30 μg $kg^{-1}$ ($n = 5$) | $0.4 \pm 0.1$ | $0.8 \pm 0.3$ | $7.9 \pm 0.6^{a}$ |
| R56865 100 μg $kg^{-1}$ ($n = 8$) | $0.4 \pm 0.2$ | $0.6 \pm 0.1$ | $8.5 \pm 0.5^{a}$ |
| R56865 300 μg $kg^{-1}$ ($n = 5$) | $0.2 \pm 0.3$ | $0.7 \pm 0.2$ | $8.4 \pm 1.3^{a}$ |
| Diltiazem 100 μg $kg^{-1}$ + R56865 100 μg $kg^{-1}$ ($n = 8$) | $0.4 \pm 0.2$ | $0.7 \pm 0.2$ | $8.4 \pm 1.0^{a}$ |

Values are expressed as means ± S.E.M.
[a] $P < 0.01$ vs. stenosis.

ately after drug administration. CP-060*S* increased left circumflex coronary artery flow to same extent as diltiazem did, while R56865 did not affect it. In addition, CP-060*S* decreased heart rate, and mean arterial pressure to a similar extent as diltiazem did, while R56865 did not affect them (data not shown). These effects of CP-060*S* or diltiazem on hemodynamics disappeared within 20 min of drug administration (data not shown). The hemodynamic variables in the vehicle group are summarized in Table 1. With partial left anterior descending coronary artery stenosis, left anterior descending coronary artery flow decreased. Heart rate, mean arterial pressure, rate–pressure product, left circumflex coronary artery flow, left ventricular end-diastolic pressure (LVEDP), and LVdP/dtmax were not affected by left anterior descending coronary artery stenosis. With pacing, heart rate was increased 80–90 beats $min^{-1}$ above baseline. Pacing slightly decreased mean arterial pressure, increased LVEDP, and did not change LVdP/dtmax. Rate–pressure product and left circumflex coronary artery flow were significantly increased by pacing. Although CP-060*S* or diltiazem affected the hemodynamics immediately after drug administration, there were no significant differences between groups in heart rate, mean arterial pressure, rate–pressure product, left anterior descending coronary artery flow, left circumflex coronary artery flow, LVEDP, or LVdP/dtmax at any of the time points indicated in Table 1 (data not shown).

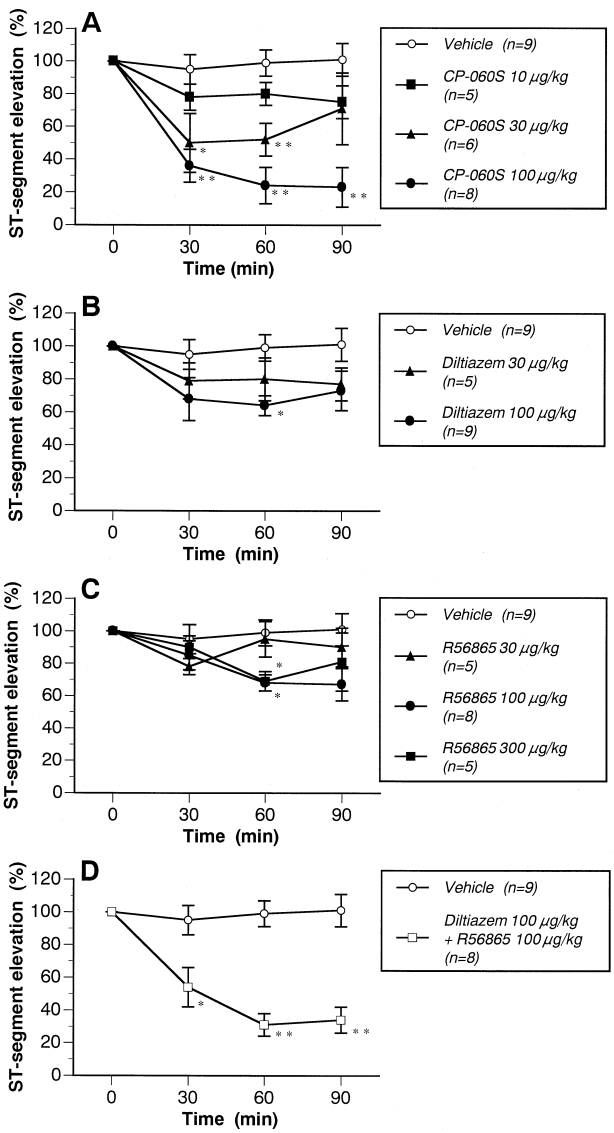


Fig. 2. Effects of (A) CP-060*S*, (B) diltiazem, (C) R56865, and (D) co-administration of diltiazem 100 μg $kg^{-1}$ and R56865 100 μg $kg^{-1}$ on pacing-induced ST-segment elevation in anesthetized dogs. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle.

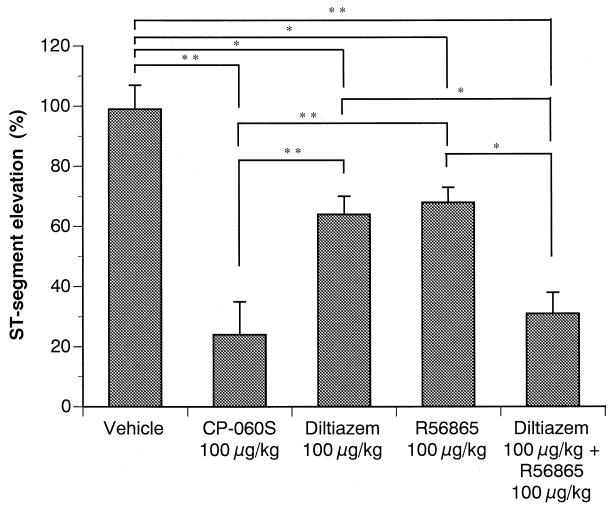


Fig. 3. Effects of the vehicle ($n = 9$), CP-060*S* 100 μg $kg^{-1}$ ($n = 8$), diltiazem 100 μg $kg^{-1}$ ($n = 9$), R56865 100 μg $kg^{-1}$ ($n = 8$), and co-administration of diltiazem 100 μg $kg^{-1}$ and R56865 100 μg $kg^{-1}$ ($n = 8$) on pacing-induced ST-segment elevation 60 min after treatment in anesthetized dogs. $^{*}P < 0.05$, $^{**}P < 0.01$.

The baseline epicardial ST-segment was similar for all groups and was not significantly altered by left anterior descending coronary artery stenosis (Table 2). When pacing was started during left anterior descending coronary artery stenosis, the ST-segment was significantly elevated to a similar extent in all groups (Table 2). Fig. 2 summarizes the effects of CP-060*S*, diltiazem, R56865, and co-administration of diltiazem 100 μg $kg^{-1}$ and R56865 100 μg $kg^{-1}$ on the pacing-induced ST-segment elevation. The ST-segment elevation induced by pacing at 30, 60 and 90 min after vehicle treatment was similar to that for control pacing. CP-060*S* significantly suppressed the ST-segment elevation at doses of 30 and 100 μg $kg^{-1}$ (Fig. 2A), while diltiazem suppressed it at a dose of 100 μg $kg^{-1}$ (Fig. 2B). Both drugs suppressed the ST-segment elevation in a dose-dependent manner. Since atrio-ventricular nodal block appeared during pacing when CP-060*S* or diltiazem was administered at doses higher than 100 μg $kg^{-1}$, 100 μg $kg^{-1}$ was the maximum permissible dose of CP-060*S* or diltiazem used in this experiment. R56865 significantly suppressed the ST-segment elevation to the same extent at

Table 3
Regional myocardial blood flow during pacing-induced ischemia in anesthetized dogs treated with CP-060*S* (100 μg kg$^{-1}$)

| | Baseline | Stenosis | Pacing | Drug | Pacing |
|---|---|---|---|---|---|
| | | | Control | | 60 min post-drug |
| *Ischemic zone* | | | | | |
| Subendocardium (ml min$^{-1}$ g$^{-1}$) | 0.667 ± 0.045 | 0.610 ± 0.048 | 0.465 ± 0.026 | 0.524 ± 0.060 | 0.500 ± 0.085 |
| Subepicardium (ml min$^{-1}$ g$^{-1}$) | 0.686 ± 0.049 | 0.669 ± 0.048 | 0.697 ± 0.046 | 0.573 ± 0.061 | 0.736 ± 0.108 |
| Subendocardial/Subepicardial flow ratio | 0.97 ± 0.01[a] | 0.91 ± 0.01 | 0.67 ± 0.01[b] | 0.91 ± 0.02 | 0.67 ± 0.02[b] |
| *Non-ischemic zone* | | | | | |
| Subendocardium (ml min$^{-1}$ g$^{-1}$) | 0.706 ± 0.058 | 0.699 ± 0.073 | 1.033 ± 0.064[b] | 0.574 ± 0.061 | 0.959 ± 0.096[b] |
| Subepicardium (ml min$^{-1}$ g$^{-1}$) | 0.635 ± 0.034 | 0.667 ± 0.068 | 1.037 ± 0.062[b] | 0.553 ± 0.060 | 0.953 ± 0.083[b] |
| Subendocardial/subepicardial flow ratio | 1.11 ± 0.04[a] | 1.05 ± 0.04 | 1.00 ± 0.03 | 1.04 ± 0.03 | 1.00 ± 0.02 |

Values are expressed as means ± S.E.M. ($n = 5$).
[a] $P < 0.05$. vs. stenosis.
[b] $P < 0.01$ vs. stenosis.

doses of 100 and 300 μg kg$^{-1}$ (Fig. 2C). Co-administration of diltiazem 100 μg kg$^{-1}$ and R56865 100 μg kg$^{-1}$ significantly suppressed the ST-segment elevation (Fig. 2D). The effects of CP-060*S* 100 μg kg$^{-1}$, diltiazem 100 μg kg$^{-1}$, R56865 100 μg kg$^{-1}$, and co-administration of diltiazem 100 μg kg$^{-1}$ and R56865 100 μg kg$^{-1}$ on the pacing-induced ST-segment elevation at 60 min after drug treatment are summarized in Fig. 3. CP-060*S* 100 μg kg$^{-1}$ significantly suppressed the ST-segment elevation at 60 min after treatment more potently than diltiazem 100 μg kg$^{-1}$ or R56865 100 μg kg$^{-1}$ (CP-060*S*, 24 ± 11% of the control value, $P < 0.01$ vs. diltiazem or R56865; diltiazem, 64 ± 6%; R56865, 68 ± 5%). Co-administration of diltiazem 100 μg kg$^{-1}$ and R56865 100 μg kg$^{-1}$ significantly suppressed the ST-segment elevation more potently than diltiazem 100 μg kg$^{-1}$ or R56865 100 μg kg$^{-1}$ alone, and to a similar extent as CP-060*S* 100 μg kg$^{-1}$ (diltiazem + R56865, 31 ± 7% of the control value, $P < 0.05$ vs. diltiazem or R56865).

Table 3 summarizes the changes in regional myocardial blood flow in the CP-060*S* 100 μg kg$^{-1}$ group. Non-ischemic regional myocardial blood flow was significantly increased by control pacing ($P < 0.01$) or 60 min post-drug pacing ($P < 0.01$), and the subendocardial/subepicardial flow ratio was unchanged. Ischemic regional myocardial blood flow was unchanged by control pacing or 60 min post-drug pacing, and the subendocardial/subepicardial flow ratio was significantly decreased ($P < 0.01$). CP-060*S* did not increase regional blood flow in the ischemic myocardium at 60 min post-drug pacing compared with control pacing in spite of causing a significant suppression of pacing-induced ST-segment elevation (from 9.5 ± 2.0 mV to 4.7 ± 1.6 mV, $P < 0.01$).

## 4. Discussion

A pacing stress test has been used in patients with angina pectoris (Feldman et al., 1996). The pacing-induced ischemia model used in the present study simulates a pacing stress test or stable angina pectoris (Grover and Parham, 1987a,b). Because the reactive hyperemia is blocked by partial left anterior descending coronary artery stenosis in this model, the left anterior descending coronary artery flow does not increase in response to the increase in myocardial oxygen demand induced by the pacing stress. Epicardial ST-segment elevation is produced as a consequence of the myocardial ischemia. Thus, ST-segment elevation has been shown to be correlated with regional myocardial blood flow (Mirvs et al., 1986). In the present study, there were no significant differences between groups in hemodynamic parameters, i.e., heart rate, mean arterial pressure, rate–pressure product, left anterior descending coronary artery flow, left circumflex coronary artery flow, LVEDP, and LVdP/dtmax, at any time point. Moreover, CP-060*S* did not increase regional blood flow in the ischemic myocardium during post-drug pacing compared with control pacing in spite of causing a significant suppression of the pacing-induced ST-segment elevation. Previous reports have shown that $Ca^{2+}$ channel blockers, e.g., diltiazem, are able to suppress the epicardial ST-segment elevation in the pacing-induced ischemia model in a manner which is independent of changes in myocardial blood flow or hemodynamic alterations (Grover and Parham, 1987a,b; Grover et al., 1989, 1990, 1995). Furthermore, R56865 and CP-060*S* have been reported to not increase regional myocardial blood flow in the ischemic zone during coronary occlusion (Vandeplassche et al., 1991a; Suzuki et al., 1998b). Therefore, these findings indicate that CP-060*S*, diltiazem, and R56865 suppress the pacing-induced ST-segment elevation independently of changes in regional blood flow in the ischemic myocardium or hemodynamic parameters, in other words, as a consequence of a direct myocardial protective action.

Diltiazem significantly suppressed the pacing-induced ST-segment elevation by 35% at a dose of 100 μg kg$^{-1}$ 60 min after treatment. Atrio-ventricular nodal block ap-

peared during pacing at diltiazem doses higher than 100 μg $kg^{-1}$, and thus, 100 μg $kg^{-1}$ was the maximum permissible dose of diltiazem in this experiment. Therefore, diltiazem suppressed the ST-segment elevation by maximally 35% as a direct cardioprotective effect. We have previously reported that the $Ca^{2+}$ channel blocking profile of CP-060*S* in the cardiovascular system in vivo is qualitatively similar to that of diltiazem (Suzuki et al., 1999). In this study, CP-060*S* and diltiazem at 100 μg $kg^{-1}$ increased left circumflex coronary artery flow to a similar extent. Thus, the $Ca^{2+}$ channel blocking activity of CP-060*S* is equal to that of diltiazem. However, CP-060*S* significantly suppressed the ST-segment elevation by 75% at a dose of 100 μg $kg^{-1}$ 60 min after treatment, superior to the effect of diltiazem ($P < 0.01$). This discrepancy seems irresolvable unless CP-060*S* has an additional effect which diltiazem does not.

It is believed that, under ischemic conditions, $Na^+$-overload occurs in the myocardium, which eventually leads to $Ca^{2+}$-overload via $Na^+/Ca^{2+}$ exchange (Tani, 1990; Ver Donck et al., 1993). Subsequently, $Ca^{2+}$-overload plays a crucial role in functional and structural damage. Recently, rapid $Na^+$-overload during myocardial ischemia has been reported and attributed to intracellular acidosis and ATP depletion (Butwell et al., 1993; Van Emous et al., 1997). Lidocaine reduces this $Na^+$-overload and the intracellular acidosis, and also protects against the ATP depletion (Butwell et al., 1993; Van Emous et al., 1997). The preservation by lidocaine of myocardial ATP levels during ischemia has been explained by the diminished consumption of ATP by the $Na^+/K^+$ ATPase, as a result of the decreased influx of $Na^+$ via $Na^+$ channels (Van Emous et al., 1997). Therefore, $Na^+$ channels play an important role in the $Na^+$-overload early in the course of myocardial ischemia. As it has been generally assumed that voltage-gated $Na^+$ channels are inactivated by the membrane depolarization that occurs during ischemia, it was thought that they could not contribute to the $Na^+$-overload. However, hypoxia (Ju et al., 1996) and the ischemic metabolite lysophosphatidylcholine (Burnashev et al., 1991) cause long-lasting bursts of opening and delay the inactivation of $Na^+$ channels. This non-inactivating $Na^+$ current plays a role in maintaining the plateau and duration of the action potential in normal cardiomyocytes (Saint et al., 1992), whereas hypoxia increases the amplitude of the current that is resistant to inactivation during depolarization (Ju et al., 1996). The non-inactivating $Na^+$ current has been proposed to be one of the pathways for $Na^+$-overload in the ischemic heart (Ver Donck et al., 1993). Lidocaine and tetrodotoxin inhibit the non-inactivating $Na^+$ current and suppress physiological $Na^+$ channel activity (Ju et al., 1996), whereas R56865 inhibits the non-inactivating $Na^+$ current without apparently suppressing physiological $Na^+$ channel activity (Ver Donck et al., 1993). As a consequence of the blockade of the non-inactivating $Na^+$ current, lidocaine, tetrodotoxin and R56865 prevent $Na^+$-, $Ca^{2+}$-overload in ischemic cardiomyocytes (Ver Donck et al., 1993; Haigney et al., 1994), whereas $Ca^{2+}$ channel blockers, e.g., diltiazem, exhibit only weak protection against $Na^+$-, $Ca^{2+}$-overload in ischemic cardiomyocytes (Ver Donck et al., 1993; Tamura et al., 1996). Although several studies have suggested that R56865 ameliorates ischemia- and reperfusion-induced myocardial damage in rats, rabbits, dogs and pigs by preventing the $Na^+$-, $Ca^{2+}$-overload as a consequence of the blockade of the non-inactivating $Na^+$ current (Garner et al., 1990; Vandeplassche et al., 1991a,b; Klein et al., 1995; Verscheure et al., 1995), the contribution of $Na^+$-, $Ca^{2+}$-overload to reversible myocardial ischemia such as occurs in angina pectoris remains to be elucidated. In the present study, R56865 significantly suppressed the ST-segment elevation to the same extent (about 30%) at doses of 100 and 300 μg $kg^{-1}$ at 60 min after treatment. Therefore, R56865 suppressed the pacing-induced ST-segment elevation by maximally 30% as its direct cardioprotective effect, i.e., a $Na^+$-, $Ca^{2+}$-overload preventive effect.

CP-060*S* prevents $Na^+$-, $Ca^{2+}$-overload and has $Ca^{2+}$ channel blocking activity (Tamura et al., 1996; Ohya et al., 1997; Suzuki et al., 1998a). Similar to R56865, CP-060*S* inhibits the non-inactivating $Na^+$ current without apparently suppressing physiological $Na^+$ channel activity, consequently preventing $Na^+$-, $Ca^{2+}$-overload (Fukazawa et al., 1997). Although several pathways for $Na^+$-overload have been proposed, e.g., $Na^+/K^+$ ATPase and $Na^+/H^+$ exchange, in addition to the non-inactivating $Na^+$ channel (Ver Donck et al., 1993), CP-060*S* does not inhibit $Na^+/K^+$ ATPase (unpublished data) and $Na^+/H^+$ exchange (Dr. Hearse, personal communication). CP-060*S* also does not inhibit $Na^+/Ca^{2+}$ exchange (Dr. Hearse, personal communication). This pharmacological profile of CP-060*S* indicates that the prevention of $Na^+$-, $Ca^{2+}$-overload is the result of the blockade of the non-inactivating $Na^+$ current. Indeed, CP-060*S* reduces this $Na^+$-overload and also protects against ATP depletion during global ischemia in isolated rat hearts (Dr. Fukuda, personal communication). In the present study, CP-060*S* 100 μg $kg^{-1}$ significantly suppressed the pacing-induced ST-segment elevation 60 min after treatment and was more potent than diltiazem or R56865 at 100 μg $kg^{-1}$ ($P < 0.01$ vs. diltiazem or R56865). Therefore, the suppression of the ST-segment elevation by CP-060*S* cannot be explained solely in terms of prevention of $Ca^{2+}$ channel blockade or $Na^+$-, $Ca^{2+}$-overload. The co-administration of diltiazem 100 μg $kg^{-1}$ and R56865 100 μg $kg^{-1}$ significantly suppressed the ST-segment elevation more potently than did diltiazem or R56865 at 100 μg $kg^{-1}$ and to a similar extent as CP-060*S* at 100 μg $kg^{-1}$ ($P < 0.05$ vs. diltiazem or R56865). This result suggests that the $Na^+$-, $Ca^{2+}$-overload preventive action and the $Ca^{2+}$ channel blocking action independently contribute to the suppression of the ST-segment elevation. Therefore, CP-060*S* may suppress the pacing-induced ST-segment elevation by the dual ac-

tion by preventing $Na^+$-, $Ca^{2+}$-overload and $Ca^{2+}$ channel blockade.

In summary, CP-060*S* suppressed ST-segment elevation, an index of myocardial ischemia, more potently than did diltiazem or R56865 in the canine pacing-induced angina model at the same dose. This anti-ischemic effect of CP-060*S* may derive from its ability to prevent $Na^+$-, $Ca^{2+}$-overload in addition to $Ca^{2+}$ channel blockade.

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